DHR International Journal Of Biomedical and Life Sciences (DHR-IJBLS), Vol. 2, Issue 1, 2012
Available online http://www.doublehelixresearch.com/DHRIJBLS

Semen evaluation in farm animals

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Abstract

Semen may be evaluated in bulls as part of the routine breeding soundness evaluation, for investigation of fertility problems and for use in artificial insemination (A.I.). The economic importance of a high breeding efficiency in farm animals emphasizes the benefit of accurate prediction of fertility of boar and bull semen. The artificial insemination (AI) studs need an objective and rapid, but inexpensive, method to evaluate ejaculates. Function of sperm plasma membrane under the hypoosmotic conditions, distribution and concentration of ions, or function of different organelles seem to correlate with the degree of the viability of spermatozoa after freezing and thawing procedures. Many in vitro techniques that stimulate sperm function through female–derived factors, such as zona and oocyte, in zona pellucida binding assay, in vitro fertilization (IVF) and production of embryos, were employed to predict the outcome of artificial insemination in the field. Still, majority of methods used for semen analysis today are both - tedious and expensive, and, in many cases confined to human bias. In order to increase the predictive power of assessment, simultaneous analysis of multiple sperm attributes, or outcomes of several laboratory assessments must be combined to look for the overall effect of several independent sperm parameters.

Key words: Bull sperm, Artificial insemination, Cryopreservation

Introduction

Artificial insemination (AI) is the first generation reproductive biotechnology that has made a profound contribution to the genetic improvement, particularly in dairy cattle. This impact would not have been possible without successful freezing of bull semen. The ideal semen analysis would be simple and effective, allowing the breeding capacity of a particular ejaculate to be predicted. A fertile ejaculate must meet certain semen parameter quality standards, such as: progressive motility, normal morphology, active energy metabolism, structural integrity and functionality of the membrane, penetration capacity and optimum transfer of genetic material. As most of bull semen were cryopreserved for future use. The process of cryopreservation represents an artificial interruption of the progress of the spermatozoon towards post-ejaculation maturation and fertilization.

The major disadvantage is that procedures involved in the cryopreservation process are harmful to spermatozoa and even the best preservation techniques to date result in about half of the sperm population that survive the freezing and thawing procedures. Change in temperature imposes changes on the composition and structure of various sperm plasma membrane domains [1], thereby

modifying their function. As it has been demonstrated, the cryopreservation makes damage on sperm membranes, cytoskeleton, motile apparatus and nucleus, alter cell metabolism [2,3]. They become very sensitive to any stresses by their environment in vivo as well as in vitro [2,3]. As a result, fertility from the AI with frozen thawed semen is poorer than that obtained with fresh semen, which can be partially compensated by inseminating greater numbers of live spermatozoa. For this reason, proper assessment of the post-thaw quality of spermatozoa is of highest interest for AI industry, since it can provide insights upon the fertilising capacity of the cryopreserved spermatozoa.

In order to be applied on a larger scale, the method has to be quick, relatively inexpensive and easily performed. The aim of the present review is to summarize the current knowledge of the methods available to assess in vitro quality of frozen-thawed bovine spermatozoa and the relationship of the outcome of these tests to the fertility in vivo, after AI with that semen.

Semen quality

The ultimate goal of semen assessment is to find one or a few parameters to predict the fertilising ability of the semen. Many different methods have been evaluated throughout the last decades, but only few methods have been adopted for practical work. Most of these studies have used light microscopic evaluations of classical sperm parameters, including sperm concentration, motility, morphology and viability.

Salisbury and VanDemark [4] proposed a model where both semen qualitative and quantitative parameters have to be considered in the equation for evaluation of male fertility potential. In fact, semen characteristics can be regarded as 'compensable' and 'uncompensable' in respect to the maximum likelihood of the conception attained by that semen. There is also a category of subfertile males that cannot be brought to normal fertility levels by including additional sperm numbers in the insemination doses, thus rendering the semen traits of such males "uncompensable". Semen may contain both compensable and uncompensable sperm traits and each to a different degree [1,5]. We must be able to identify sperm characteristics that prevent availability of sperm for fertilization (compensable traits) and identify sperm traits associated with incompetent fertilising sperm; that is, the sperm that can initiate, but not complete the fertilization process or sustain early embryogenesis (uncompensable traits). This subject, regarding compensable and uncompensable semen quality traits has been studied and reviewed by Saacke et al., [5], den Daas [6].

Sperm in the ejaculate are very heterogeneous in their attributes, including fertilizing capacity. Quality of spermatozoa that participate in the fertilization may not necessarily represent the whole ejaculate. Meaning that ejaculate which contains numerous subpopulations of sperm, individual numbers of which are prepared to fertilize an oocyte at different times but have very short life after initiation of their terminal attempt at fertilization. In the female, a very effective mechanism to control the number and the quality of spermatozoa that will finally compete in fertilization exists. The quantity of semen reaching the site of fertilization is reduced in relation to the number of spermatozoa inseminated. Reduction of sperm numbers on their passage through the female genital tract is of major importance, since it represents the first mechanism to reduce or abolish the polyspermia. The final barrier against spermatozoa with certain traits participating in the fertilization is the vestments of the ovum – zona pellucida. Insemination with too low number of viable spermatozoa results in reduced pregnancy rates or no pregnancy at all.

Semen evaluation techniques

From a biological point of view, only viable spermatozoa carrying intact genetic information are potentially fertile and therefore, most of the methods used so far focus on sperm viability and DNA integrity. Motility and gross morphology estimated by light microscopy are by now most used parameters for semen quality assessment, especially in AI laboratories. Due to the simplicity of the evaluation technique motility is probably the most often used criterion for routine semen evaluation. Motility may be divided in quantitative motility (percentage of sperm cells with a progressive motility) and qualitative motility. The latter involves several different parameters, some of which are the speed of the moving sperm cells, altitude of head displacement and movement pattern (circular vs. linear movement, total distance vs. progression etc.). The accuracy in terms of repeatability of this test is, however, low, and very dependent on the ability of the operator [3]. Probably for this reason, reports on the relationship between subjectively-assessed sperm motility and fertility is inconsistent [7,8]. Computer assisted semen analysis (CASA) is objective method that gives extensive information about the kinetic property of the ejaculate based on measurements of the individual sperm cells. Using CASA, motility and movement characteristics of spermatozoa have been correlated to in vivo fertility [9,10,11].

Energetic exchange (ATP concentration)

Energy for flagellar action is metabolized by the mitochondrialdense mid-piece and these combine to propel the sperm head, carrying the male haplotype, to the ovum. Flagellar motion is the main energy-demanding process of viable spermatozoa [12], with most energy derived from the hydrolysis of adenine triphosphate (ATP) to adenine di and mono phosphates [13]. The concentration of ATP in semen is related to the number of motile spermatozoa [14], which means that measurement of ATP concentration may provide an objective method of estimating sperm viability. However results concerning correlations between sperm ATP concentrations and fertility have been contradictory [15,16,17].

Sperm morphology

The assessment of sperm concentration and morphology is based on the direct relation between the incidence of abnormal spermatozoa and the type of certain morphological defects with the in vivo fertility of the bull [18]. Accurate morphological screening of the ejaculates allows elimination of bulls with a potential low fertility, prior to the entrance of bulls a progeny testing program and the preservation of semen, thus contributing to a major savings for AI enterprises. There is undoubtedly a correlation between motility and fertility as well as for morphology and fertility, provided there is a wide range of variation in the quality of the parameters assessed as well as fertility obtained with that semen. However, these correlations are reduced concomitantly with an increase in the lower limit set to accept an ejaculate for further processing. When the acceptable range for these parameters is narrow, motility and gross morphology only have limited value for separating ejaculates in respect to the expected fertility of the ejaculate.

Determination of sperm morphology by light microscopy by human eye suffers from subjectiveness, with different technicians often achieving different results on the same series of smears [2]. Increasingly, computeraided sperm head morphometry analysis (ASMA) is being evaluated and applied in veterinary medicine [19,20,21]. Computerized methods focus on evaluating the measurements that are able to quantify and classify sperm morphology correctly and offers repeatable and objective method of assessing bull sperm head morphometry within and between technicians. Measurements of sperm head variables such as area, length, width and perimeter have shown promise in the computerized analysis of bull

sperm head morphology [22]. So far, information regarding sperm head morphometry and fertility of bull semen after AI is sparse. It has been observed, however, that certain parameters regarding texture of the digitized image of the sperm head correlates with in vivo bulls' fertility, expressed as non-return ranks [23].

Sperm chromatin structure

There are many factors leading to production of abnormally shaped spermatozoa. Various toxic agents and deviations in scrotal temperature affect different endpoints in the formation of morphologically abnormal sperm. Abnormal chromatin structure may lead to problems in packaging of sperm nuclear material possibly related to morphologically abnormal spermatozoa [22]. Morphological shapes of spermatozoa determined by visual examination of the sperm in light microscopy and visually classifying the cells as either normal or abnormal were low or inconsistent with abnormal sperm chromatin structure [3]. Abnormal sperm chromatin structure may be assessed by Sperm Chromatin Structure Assay (SCSA), which defines abnormal chromatin structure as susceptibility of DNA to denaturation in situ [22,23]. In the SCSA, whole spermatozoa, or sonication-released nuclei are either heated or treated with HCl to denature DNA in situ and then stained with the metachromatic dye acridine orange (AO). Suspension of stained cells is run through flow cytometer and excited with blue laser light, AO intercalated into native, double stranded DNA fluoresces green, whereas AO associated with single-stranded DNA fluoresces red. Thus, a shift from green to red fluorescence corresponds to the DNA denaturation. The extent of DNA denaturation quantified by the SCSA has been shown to be sensitive indicator of male fertility potential in various animal species and also in human [22]. Compared to visual estimation of sperm morphological abnormalities, SCSA is a very sensitive test. Karabinus et al. [2] showed that chromatin structural changes in spermatozoa after scrotal insulation of Holstein bulls could be detected 3 days after heat stress, whereas light microscopic observations did not detect abnormalities until 11 days [1].

Assays of plasma membrane integrity

The sperm plasma membrane is the primary site where lesions occur during freezing-thawing of semen [6,7]. An intact and functionally active membrane is essential for the spermatozoon to sustain metabolism, undergo capacitation and acrosome reaction (AR) and, further, attach to and penetrate the oocyte zona pellucida (ZP) [21,9]. It has been recognized during the last several decades that one of the major features discriminating dead from live cells is a loss of the transport function and physical integrity of the plasma membrane. Based on this phenomenon, a plethora of assays of cell viability has been developed. For example, since the intact membrane of live cells excludes a variety of charged dyes, such as trypan blue or propidium iodide (PI), incubation with these dyes results in selective labeling of dead cells, while live cells show no or minimal dye uptake. A combination of supravital staining dyes such as trypan blue/giemsa, eosin/aniline blue and some other classical dyes are widely used for differential live/dead staining of fresh ejaculated spermatozoa.

For light microscopic evaluation a relatively high concentration of the dye (in mg/ml) is required. At these concentrations, eosin, and many other dyes are toxic, which can lead to underestimation of the proportion of live cells [8,1,6]. Moreover, these dyes are used nearly exclusively to stain fresh ejaculated spermatozoa, because glycerol, a most extensively used cryoprotectant, interferes with the staining. Today assays for exclusion of fluorescent dyes are the most popular for evaluation integrity of frozen-thawed sperm plasmalemma followed by examination with fluorescence microscopy or flow cytometry [3,8]. Microscopic evaluation enables direct

observation of compartmentalization of dyes in target organelles or membrane domains of spermatozoa, however flow cytometric analysis is based on quantitative analysis of events (cells) that emit certain wavelength light after being excited by a laser beam. Still the latter technique has proven advantageous over microscopic assessment, allowing the examination of thousands of cells over a much shorter period than it is needed for microscopy [8].

Vital staining of spermatozoa

Once damaged, spermatozoa are not able to reseal the compromised plasmalemma [6,1], and therefore, cannot maintain those ion and co-factor concentrations essential to sperm survival. The development of staining technology using fluorophores for nucleic acid, intracytoplasmic enzymes, or membrane potential has provided with new tools for assessing the functionality of frozen-thawed spermatozoa. Single fluorophores or in combinations can be used determining sperm membrane integrity.

The most commonly used classic nucleic acid stains are bisbenzimidazoles Hoechst 33258 (H258) and Hoechst 33342 (H342) and phenanthridines, such as ethidium bromide (EtBr), propidium iodide (PI) and ethidium homodimer (EthD-1). Bisbenzimide dyes are water-soluble minor groove binding AT-selective DNA stains and are relatively non-toxic. There are, however, several drawbacks that make bisbenzimides less attractive for semen analysis. The dyes are semi-permeant to intact membranes, and have extremely broad emission spectra that overlap that of other dyes, and therefore, require several filters for microscopical evaluation of combined staining. Moreover, UV light needed for the excitation of the cell might be harmful for the cells analyzed as well as the examiner.

Under illumination, SYBR-14 loaded viable sperm cells fluoresce bright green, whilst damaged cells take-up PI and fluoresce red. The relationship between SYBR-14/PIstained and flow cytometrically-assessed semen viability post-thaw and in vivo fertility obtained with that semen is controversial. Some trials resulted in no significant correlations with field fertility [6,1], whereas promising results have been obtained in other studies [11].

Osmotic resistance test (hypo-osmotic swelling test)

Functional integrity of the plasma membrane can also be evaluated by measuring the resistance of sperm membranes to swelling in a hypo-osmotic medium. This much simpler method is based on the ability of the membranes to allow passage of water in order to establish equilibrium between the fluid compartment within the spermatozoon and the external surroundings [20,21,25,1]. It was suggested that the ability of spermatozoa to swell in the presence of hypo-osmotic medium reflects normal water transport across the sperm membrane, which is a sign of normal membrane integrity and functional activity [20]. The functionally active spermatozoa exposed to a hypo-osmotic stress swell due to the influx of water and subsequently increase in volume to establish the equilibrium between the cytosol and the extracellular milieu. Spermatozoa with compromised or inactive membranes are unable to regulate water influx and remain not swollen. Thus, hypo-osmotic swelling tests may be useful in assessing changes in the sperm membrane functional integrity during freezing thawing procedures [15]. Attempts have been made to correlate sperm plasma membrane integrity to fertility, but great variation is seen between studies and methods used [7,1].

Analysis of multiple sperm attributes

As mentioned above, spermatozoa need to possess certain attributes in order to achieve fertilization [1]. It is unlikely that a process so complex as fertilization could be predetermined by a single parameter of sperm quality. It is

agreed that spermatozoon is multifunctional cell that must possess a large number of attributes that make it potentially fertile. Any spermatozoon lacking any of these attributes or not possessing enough of an attribute will be infertile. Because sperm require many attributes, an assay, measuring a single attribute will fail to detect defective spermatozoa, and will have low power to predict which samples are likely to possess low, or high fertility. So far, the analysis of a single sperm viability parameter cannot predict the outcome of a process as complex as fertilization. It has been shown that combination of several post-thaw sperm quality attributes, as compared to any single sperm quality trait, can explain more variation in fertility between the bulls [4]. For this reason, in order to increase the predictive power of the test, simultaneous analysis of multiple sperm attributes [3], or outcomes of several laboratory assessments can be combined statistically to look for the overall effect of several independent sperm parameters [22,3,19].

Summary

Although many efforts have been made, so far there are no currently available methods, or sperm assessment techniques that could accurately predict sperm fertilizing potential. Artificial insemination (AI) has proven to be the most effective tool for genetic improvement of animals of zootechnical importance, especially in the cattle industry. The inspection and handling of semen is considered a key and essential step for assessing fertility and the successful use of semen. Knowledge of fertility in bulls is an objective of great importance for the production of semen, which is achieved by good analysis of the semen, among other assessments. To design an ideal bovine semen analysis to properly assess and predict the fertility of an ejaculate is the goal of many specialists in the reproduction field. If successful, such a method could contribute to improved herd fertility. It could also result in major savings for AI enterprises, for the bulls with inferior fertility could be selected and culled prior entering the fertility and progeny testing programmes. All current work on semen analysis seeks to identify some kinetic, morphological or biochemical parameters indicating the status of the sperm cell at any given time, while at the same time correlating with fertility and ejaculate quality. In routine production, the test should be accurate, simple, fast and economical.

Most promising tests available are sperm chromatin structure assay (SCSA) and sperm viability assessed by flow cytometry, however the present review has shown that today we still do not have single in vitro sperm quality assessment method that can accurately predict sperm fertilizing potential. In order to increase the predictive power of the test, assessment of several sperm attributes must be combined in a simultaneous analysis, or outcomes of several laboratory assessments must be combined statistically to look for the overall effect of several independent sperm parameters.

Acknowledgment

Author Arvind Singh like to thanks Department of life Sciences, Venkateshwara University, J.P. Nagar, U.P., India for financial support in reviewing subject.

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